

## Review Article

# Molecular genetics of cardiomyopathy: changing times, shifting paradigms

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### Summary

Congestive heart failure is a major problem in developed and developing countries alike. Primary dysfunction of the heart muscle accounts for a significant proportion of patients with a non-ischaemic cause of heart failure. Application of genetic techniques has facilitated identification of some molecular causes of the inherited form of these diseases, dramatically increasing our understanding of the pathogenesis of these primary, previously termed 'idiopathic', cardiomyopathies over the last few decades. Knowledge of the different causes is beginning to coalesce into aetiological principles underlying the clinically distinguished cardiomyopathies. Hypertrophic cardiomyopathy (HCM) now appears to be a disease

caused by a dysfunctional sarcomere, dilated cardiomyopathy (DCM), a disease of myocytic structural instability, and arrhythmogenic right ventricular cardiomyopathy, a disease of accelerated myocyte death. The aetiology of both HCM and DCM probably also involves cardiac energy imbalances, while additional factors modify the clinical expression in all cardiomyopathies. Even though our knowledge of the genetic aetiology of the cardiomyopathies is still incomplete, it already has relevant clinical significance. Elucidation of the full genetic contribution to the development and progression of the cardiomyopathies represents a new challenge in the study of these diseases, and will undoubtedly lead to new therapeutic approaches in the not-too-distant future.

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Congestive heart failure is a major problem in developed and developing countries alike. In Europe, 50 million of the population of 1 000 million suffer from heart failure,<sup>1</sup> and in the USA, 4.9 million patients are treated for heart failure each year.<sup>2</sup> Although it is difficult to obtain similar statistics for South Africa, figures obtained from the Medical Research Council report that deaths from all forms of cardiovascular disease (CVD) account for about 22% of total mortality.

Heart failure entails breakdown of the usually efficient cardiac pumping mechanism and consequently failure to meet the variable demands of the body's tissues, and may be either ischaemic or non-ischaemic in origin.<sup>3–6</sup> Primary dysfunction of the heart muscle (cardiomyopathy) accounts for a significant proportion of patients with a non-ischaemic cause of heart failure.<sup>7</sup> This dysfunction springs from active remodelling of the myocardial structure of either one or both ventricles, in an attempt to normalise an underlying fault in

pump function. The remodelling usually involves hypertrophy of the individual myocytes, which may extend to the whole ventricular myocardium and which may, for reasons not completely clear, progress to cardiac dilation.<sup>8,9</sup> Although at first either of these types of myocardial change may be beneficial, they become maladaptive with time. Both hypertrophy and dilation place greater energy demands on the heart, and progressively escalate systolic and/or diastolic dysfunction, placing the heart on a downward spiral towards complete heart failure.

Originally, in the absence of aetiological clues, the cardiomyopathies were grouped together as 'idiopathic' cardiomyopathies and were sub-classified, by morphological and haemodynamic characteristics, into five categories. These were hypertrophic, dilated, arrhythmogenic right ventricular and restrictive cardiomyopathy, as well as the broad category of the unclassified cardiomyopathies.<sup>10</sup> These groupings are still convenient, although with increasing understanding of the molecular basis of the inherited cardiomyopathies, few of them remain idiopathic, and we also

now know that aetiological overlap occurs in these clinically differentiated disorders. New insights have been gained most speedily and extensively for hypertrophic cardiomyopathy (HCM), followed by dilated cardiomyopathy (DCM), while much still remains to be learnt about arrhythmogenic right ventricular cardiomyopathy (ARVC) and familial forms of restrictive cardiomyopathy (RCM).

## Characteristic features of the different cardiomyopathies

### Clinical features

The distinguishing clinical, histological and symptomatic features of the four categories of idiopathic cardiomyopathies are summarised in Table I.

HCM features all aspects of a failing heart that has remodelled according to the hypertrophic route. The disease is generally characterised, morphologically, by hypertrophy

**TABLE I. SUMMARY OF MORPHOLOGICAL, CLINICAL AND SYMPTOMATIC FEATURES OF THE FOUR CARDIOMYOPATHIES**

<i>Cardiomyopathy</i>	<i>HCM</i>	<i>DCM</i>	<i>ARVC</i>	<i>RCM</i>
Ventricles	Left mostly Hypertrophy of ventricular wall	Left mostly Dilation of ventricular chamber	Right mostly Infiltration of RV free wall by fibro-fatty tissue Normal or thinned RV wall ± RV dilation	Both Non-dilated, Non-hypertrophied, Non-compliant
Variants	Partial occlusion of ventricular chamber Asymmetrical Concentric Apical Mid-cavity DCM-like Old age	Normal or thinned ventricular walls  With or without conduction defects	Infiltration Replacement of myocytes in RV free wall	Amyloid Other
Atria	LA dilation	LA or bi-atrial dilation	RA dilation	Bi-atrial dilation
Haemodynamic function	Reduced diastolic	Reduced systolic & diastolic	Some reduced systolic & reduced diastolic	Severely reduced diastolic
Electrophysiology	Ventricular arrhythmias	Ventricular arrhythmias ± conduction defects	Ventricular tachyarrhythmias ± conduction defects	AV block
Histology	Pathological hypertrophy  Myocytic disarray	Apoptosis Fibrosis Hypertrophic & atrophic fibres	Infiltration by fibro-fatty tissue Inflammation	Amyloidosis Ischaemic damage
Symptoms	Dyspnoea, syncope, angina, palpitations, embolism, CHF	Fatigue, exercise intolerance, angina, CHF	Syncope, palpitations	Systemic and pulmonary venous congestion, fibrillation
Prevalence	1:500	1:2 500	1:5 000 (Italy)	Rare
Mode of inheritance	Autosomal dominant	Autosomal dominant, autosomal recessive, X-linked	Autosomal dominant	Possibly autosomal dominant
Familial rate	>50%	~30%	Unknown	Unknown
Mode of death	Mostly SUD	SUD CHF	SUD	SUD CHF
Phenocopies	Noonan's syndrome Friedreich's ataxia VLCAD deficiency	Skeletal myopathies Limb-girdle muscular dystrophies Barth syndrome	Naxos disease	Autosomal dominant familial amyloidosis

LV = left ventricle, RV = right ventricle, LA = left atrium, RA = right atrium, SUD = sudden unexpected cardiac death, CHF = congestive heart failure, AV = atrio-ventricular, VLCAD = very-long-chain Acyl-CoA.

that most often affects the left ventricle and interventricular septum and usually develops fully between puberty and the third decade of life.<sup>11,12</sup> HCM is extremely variable in terms of its clinical presentation, the amount and location of hypertrophy, and the risk of sudden cardiac death.<sup>13</sup> Depending on the location of hypertrophy, HCM is subdivided into numerous hypertrophic variants, some of which are now proposed to be associated with specific genetic defects.<sup>14</sup> Electrocardiographically, the most important feature in terms of outcome is ventricular tachyarrhythmias that can be life-threatening, while left ventricular hypertrophy with or without ST-T waves and Q-wave changes is important in the diagnosis of the condition.<sup>15</sup>

DCM, on the other hand, is the classic example of a heart remodelled in keeping with the dilation route. In this disorder, morphologically, all cardiac chambers are usually enlarged, and the dilation can occur in the presence of normal or thinned cardiac walls, with concomitant systolic and diastolic dysfunction. Electrophysiologically, these dilated hearts are also prone to ventricular arrhythmias, which can, as with HCM, lead to sudden cardiac death.<sup>10</sup>

In ARVC, it is most often the right ventricle that is affected by infiltration of the ventricular wall, or replacement of the myocytes in this wall by a fibro-fatty tissue.<sup>16,17</sup> These hearts are prone to premature beats and ventricular tachycardia, which may be provoked by exercise-induced catecholamine release and thus may cause sudden cardiac death during physical activity.<sup>16</sup>

In RCM, both ventricles are non-compliant, causing end-diastolic pressures to increase and both atria to dilate. In primary, idiopathic RCM the ventricles are neither dilated nor hypertrophied; however, secondary RCM may be due to infiltration of the heart muscle by amyloid or sarcoid fibrils, or can be a feature of late stages of DCM, HCM, hypertensive, valvular and ischaemic heart disease.<sup>18</sup> These infiltrative forms of RCM may be accompanied by increased wall thickness. Atrial fibrillation due to atrial dilation is a common feature of RCM.<sup>18</sup>

## Diagnosis

Clinical diagnosis of the cardiomyopathies relies extensively on techniques that allow measurement of functional parameters and visualisation of macroscopic and microscopic morphological features of the heart. HCM is therefore diagnosed when ventricular wall thickness is equal to or exceeds 13 mm on echocardiography in the absence of another cause such as hypertension or aortic stenosis.<sup>19</sup> DCM is diagnosed when there is impaired systolic function (ejection fraction < 45% or fractional shortening < 25%) and left ventricular cavity size > 112% of predicted normal values.<sup>20</sup> In most cases of ARVC, the disease can only be diagnosed by elaborate investigation, involving family history, electro- and echocardiography, right ventricular angiography and contrast ventriculography, and histological examination of the right ventricular free wall. Because of this difficulty in diagnosis, the present diagnosis for ARVC, in the absence of a histological finding of fibro-fatty infiltration of the right ventricular myocardium, requires that a patient demonstrate

either two major, one major plus two minor, or at least four minor diagnostic criteria.<sup>16,21</sup> Primary RCM is diagnosed on the basis of restrictive filling and reduced diastolic volume of either or both ventricles with normal systolic function and wall thickness, in the absence of another cause. The features may be found on echocardiography, but cardiac catheterisation and endomyocardial biopsy are required for diagnosis.<sup>10,18,22</sup>

## Histology

As an adjunct to clinical diagnosis, the four types of primary cardiomyopathy can also be distinguished on histological examination of endomyocardial biopsy. However, diagnosis by endomyocardial biopsy may not be definitive in all cases. Specifically, absence of abnormal histological findings can be due to the segmental nature of myocardial involvement in the cardiomyopathies.

HCM is characterised by pathological hypertrophy of the individual myocytes, but the characteristic feature of HCM is myocytic and myofibrillar disarray. This disturbance of the normally extremely ordered cardiac syncytium, present to a lesser extent in other cardiac disorders as well as in normal hearts, has been found to affect up to 30% of total tissue studied in HCM hearts.<sup>23</sup> In DCM, the histology is usually non-specific, showing mild interstitial fibrosis, some degree of myocardial cell degeneration and apoptosis, while myocyte hypertrophy is uniform and disarray absent.<sup>9,24</sup> The frequency of 'ghost' myocytes, lacking myofibrillar elements, has been correlated with the degree of dilation and severity of symptoms in DCM.<sup>25</sup> For ARVC diagnostic purposes, infiltration of the myocardium by fibrous tissue should be seen in more than 3%, and by fatty tissue in more than 40%, of biopsy sections; there are also usually signs of inflammation and areas of apoptosis.<sup>16,26</sup> In RCM, pericellular fibrosis is evident, while there may be some evidence of myocyte hypertrophy, attenuation and degeneration.<sup>18</sup> Additionally, histology is vital in the diagnostic work-up of patients with RCM to exclude amyloidosis, haemochromatosis and sarcoidosis.

## Sudden cardiac death

Although there is symptomatic overlap between these four categorised disorders (Table I), they can and should be distinguished by the cardiologist for appropriate management of patients. However, these cardiomyopathies are highly variable in their clinical presentation. Many patients remain asymptomatic for years and consequently only seek medical attention when the condition is quite advanced. Frighteningly, these cardiomyopathies often result in sudden cardiac death, frequently among the young, asymptomatic and apparently healthy and health-conscious, with the concomitant shock, grief and regret amongst those that remain. In fact, worldwide, HCM is considered to be the most common cause of sudden cardiac death among young, healthy individuals and athletes,<sup>27</sup> except in the Far East and in Italy, where it is reported that 20–25% of these deaths are caused by either idiopathic ventricular tachycardia or ARVC.<sup>28,29</sup>

Frequently, it is only enquiries initiated by these sudden deaths that trigger, in both the clinician and the family, an awareness of the possible familial nature of the disease and, consequently, additional at-risk family members may be identified at an earlier stage.

## Genetics of the cardiomyopathies

### Lessons from genetics

Our understanding of the molecular aetiologies of the cardiomyopathies appears to be commensurate with the availability of large families in which the disease segregated through multiple generations, which facilitated molecular genetic investigations of the underlying cause, at least in the inherited forms of these disorders. These studies have also lead to a greater awareness of the subtle clinical manifestations and occurrence of these diseases in the general population. Therefore, although HCM used to be considered quite rare,<sup>24</sup> it is now recognised to be one of the most common inherited cardiac disorders, with a prevalence rate (1:500) similar to that estimated worldwide for the common inherited disease, familial hypercholesterolaemia.<sup>30</sup> Also, knowledge of the genetic defects that underlie some of the inherited cardiomyopathies has allowed DNA-based screening for mutation-carriers, and a clearer picture of disease penetrance (the risk of development of clinical disease in these individuals) as well as the mode of inheritance has emerged (Table I). The identification of numerous clinically unaffected mutation-carriers has also lead to the realisation that many more cases of cardiomyopathy are familial than was originally thought, that the clinical manifestation of the disease is modified by additional factors, and that mortality figures attributable to cardiomyopathy are probably lower than at first estimated.

In addition, besides the classic cardiomyopathies discussed above, numerous syndromic diseases exist in which a form of cardiomyopathy is merely one of many clinical features (Table I). These phenocopies of the cardiomyopathies had generally been dismissed as unlikely to provide clues in the search for the molecular cause of the pure cardiomyopathies. However, recently, elucidation of the molecular lesions underlying some syndromes of which cardiomyopathy is a feature has added tremendously to our understanding of the underlying pathophysiological principles, which may also be applicable to the non-inherited forms of the different cardiomyopathies.

### HCM – a ‘sarcomeropathy’...

The large families with multiple individuals unequivocally affected with uncomplicated HCM, as described in the early to mid 1900s,<sup>31,32</sup> made this cardiomyopathy most amenable to molecular genetic analysis. So, over the last 10 years, and at an ever-increasing rate, more and more evidence indicated that HCM is a ‘sarcomeropathy’.<sup>33</sup> Currently, it is known that many cases of sporadic and familial HCM are caused by more than 150 distinct mutations in at least nine different genes encoding protein components of the

contractile unit of cardiac muscle, the sarcomere (Table II, Fig. 1) (FHC database). This knowledge has been useful in explaining many clinical features of the disease and has also become a valuable adjunct to clinical diagnosis, management and counselling of HCM-affected patients. Probably the most useful corollary of aetiological understanding was the discovery of correlations between specific genetic defects and the clinical outcome. Significantly, these defects appeared to correlate better with disease prognosis than did any clinical parameter tested to date, and this was applicable to carriers of any age. Some mutations were associated with normal life expectancy, while others were associated with a high risk of sudden cardiac death.<sup>34,35,39</sup> It also became clear that, although extreme hypertrophy is still a predictor of poor prognosis,<sup>36</sup> hypertrophy in general, and risk of sudden cardiac death are unrelated features (Fig. 2).<sup>35,39</sup> In fact, it was found that defects in some of these sarcomeric protein-encoding genes, e.g., troponin T (Fig. 1), often cause minimal hypertrophy (Fig. 2a), yet are associated with early sudden cardiac death (Fig. 2b).<sup>35,37–39</sup> In a South African study, it was found that this was especially true in young male carriers of the troponin T R92W mutation.<sup>35,39</sup> Significantly, this mutation shows a founder effect, i.e. it is enriched in the South African population due to sub-population history.<sup>40</sup>

In addition, there have been indications that some of the morphological variants of hypertrophy (Table I) are associated with specific genes or mutations (Table II). For instance, the troponin T R92W mutation has been associated, in Japanese patients, with the DCM-like variant with early cardiac decompensation and progression from hypertrophy to dilation.<sup>41</sup> Other, sometimes weaker, associations have been demonstrated between particular defects and the apical variant (troponin I, Fig. 1),<sup>42</sup> or the mid-cavity variant (myosin light chains, Fig. 1),<sup>43</sup> or the variant in which hypertrophy does not stop after the third decade, but progresses throughout life, akin to the hypertrophy of old age (myosin binding protein-C, Fig. 1).<sup>44,45</sup>

Studies of the functional effects of these mutations indicated that the encoded faulty proteins become ‘poison peptides’ that disrupt the function and the structure of the sarcomere, and may directly give rise to extensive myofibrillar and myocytic disarray, characteristic of HCM.<sup>46,47</sup> These functional studies also revealed that most HCM-causing defects result in abnormal calcium ( $\text{Ca}^{2+}$ ) sensitivity of contractility,<sup>48</sup> supporting the earlier observation of altered  $\text{Ca}^{2+}$  handling by HCM hearts.<sup>49,50</sup> In addition, it was found that some defects give rise to myofibres that are hypercontractile, fitting the early ‘pre-gene era’ observation of apparent hypercontractility in HCM.<sup>51</sup> Yet, other defects give rise to myofibres that are hypocontractile,<sup>48</sup> which begs the question: ‘how can both hypo- and hypercontractile fibres produce essentially the same clinical entity?’. Moreover, any aetiological connection between phenocopies of HCM, which do not feature sarcomeric disruption, and classic HCM remained elusive until last year, when mutations in the 5'-activated AMP protein kinase (AMPK) gene were found in individuals featuring HCM and Wolf-Parkinson-White syndrome (HCM+WPW).<sup>52</sup>



TABLE II. MOLECULAR CAUSES OF THE CARDIOMYOPATHIES.

<i>Cardio-myopathy</i>	<i>Chromosome</i>	<i>Gene product (gene symbol)</i>	<i>Cellular location/function</i>	<i>Clinical phenotype</i>	<i>Reference</i>
HCM	14q11-12	Cardiac $\beta$ -myosin heavy chain (MYH7)	Sarcomere (thick filament)	Variable hypertrophy, variable SUD	39, 93, 94
	1q32	Cardiac troponin T (TNNT2)	Sarcomere (thin filament)	Minimal hypertrophy, high risk of SUD, fast progression to DCM-like variant	35, 41
	11p11.2	Cardiac myosin binding protein-C (MYBPC3)	Sarcomere (thick filament)	Progressive hypertrophy (old age-variant), more HF than SUD	45, 95
	15q22	$\alpha$ -tropomyosin (TPM1)	Sarcomere (thin filament)	Variable, but usually good prognosis; some progress to DCM-like variant	96, 97, 98
	19q13.4	Cardiac troponin I (TNNI3)	Sarcomere (thin filament)	Apical variant, old-age variant, some progress to DCM-like variant	42, 45, 99
	12q23-24	Ventricular myosin regulatory light chain (MYL2)	Sarcomere (thick filament)	Some demonstrate mid-cavity variant	43, 100
	3p21	Myosin essential light chain (MYL3)	Sarcomere (thick filament)	Some demonstrate mid-cavity variant	143, 101
	15q14	Cardiac actin (ACTC)	Sarcomere (thin filament)	Rare	102
	2q31	Titin (TTN)	Sarcomere	Rare	103
	14q11-12	Cardiac $\alpha$ -myosin heavy chain (MYH6)	Sarcomere (thick filament)	Rare; old-age variant	45
	7q35	Cardiac 5'-AMP activated protein kinase (PRKAG2)	Enzyme, senses falling ATP levels	+ Wolff-Parkinson-White syndrome, glycogen storage disease	52, 56
DCM	Xp21	Dystrophin (DMD)	Intracellular cytoskeleton	$\pm$ Duchene's or Becker's muscular dystrophies, rapid progression to HF	62, 63
	2q35	Desmin (DES)	Intracellular cytoskeleton	+ Desmin myopathy	68, 104
	5q33-34	$\delta$ -sarcoglycan (SGCD)	Cell membrane, extracellular matrix	+ Limb girdle muscular dystrophy 2F, early onset dilation	65, 105
	6p24	Desmoplakin (DSP)	Desmosomal junction	+ Keratoderma and woolly hair	106
	1q21.3	Lamin A/C (LMNA)	Inner nuclear membrane	Often with conduction defects, $\pm$ Emery-Dreifuss muscular dystrophy, limb girdle muscular dystrophy 2B	67, 107
	Xq28	Emerin (EMD)	Inner nuclear membrane	+ Emery-Dreifuss muscular dystrophy	66
	1q32	Cardiac troponin T (TNNT2)	Sarcomere (thin filament)	Early dilation	108, 109
	14q11-12	Cardiac $\beta$ -myosin heavy chain (MYH7)	Sarcomere (thick filament)	Early dilation	108
	2q31	Cardiac titin (TTN)	Sarcomere (M-line-Z-disk)	Rare	72
	15q14	Cardiac actin (ACTC)	Sarcomere (thin filament)	+ Nemaline myopathy	71
	15q22	$\alpha$ -tropomyosin (TPM1)	Sarcomere (thin filament)	+ Nemaline myopathy	110
	Xq28	Tafazzin (G4.5)	Enzyme, produces glycerophospholipid of inner mitochondrial membrane	+ Barth syndrome, infantile onset	77
	9q13-22	Unknown	Unknown	Incomplete penetrance	111
	10q21-23	Unknown	Unknown	Mitral valve prolapse	112
	2q14-22	Unknown	Unknown	Frequent ventricular tachycardia	113
	3p22-25	Unknown	Unknown	Sick sinus syndrome and stroke	114
	6q23	Unknown	Unknown	+ Adult onset limb-girdle muscular dystrophy and conduction defects	115
	6q23-24	Unknown	Unknown	+ Juvenile sensorineural hearing loss	116
ARVC	17q21	Plakoglobin	Desmosomal junction	+ Naxos disease	83
	1q42	Cardiac ryanodine receptor	Regulates $\text{Ca}^{2+}$ release from sarcoplasmic reticulum	Particularly high risk of sudden death upon exercise (ARVC2)	80
	2q32	Unknown	Unknown	Unknown	117
	3p23	Unknown	Unknown	Unknown	118
	14q12-22	Unknown	Unknown	Unknown	119
	14q23-24	Unknown	Unknown	Unknown	120
	10p12-14	Unknown	Unknown	Unknown	121

HF = heart failure; SUD = sudden cardiac death.

### ... and an energy-deficiency disorder?

The AMPK enzyme acts as the fuel gauge of the myocyte, sensing when adenosine triphosphate (ATP) levels in the extremely energy-sensitive myocyte run too low, and activating molecular pathways that lead to increased energy production.<sup>53,54</sup> Although mutations in different subunits of AMPK are also associated with features of glycogen storage disease, so that HCM+WPW may represent yet another phenocopy of primary HCM, they are invariably associated with muscle hypertrophy.<sup>55,56</sup> This has led to the proposal that the common underlying aetiological principle of cardiac hypertrophy, whether in HCM or in HCM-phenocopies, relates to an inequality in energy supply and demand.<sup>52</sup> In HCM, both hypercontractile and hypocontractile fibres waste energy, directly by overactivity, or by creating drag on unaffected fibres, respectively. Similarly, in the HCM-phenocopy diseases such as mitochondrial mutation-related disorders,<sup>57</sup> Friedreich's ataxia<sup>58</sup> or very-long-chain acyl-CoA dehydrogenase (VLCAD) deficiency,<sup>59</sup> the underlying mechanism relates to ineffective energy production in the mitochondria, and in HCM+WPW the sensing mechanism that should normally activate energy-producing pathways is defective.<sup>52,56</sup> Moreover, it can be speculated that the aetiological principle underlying cardiac hypertrophy in hypertension may well be the same, as greater resistance in the vessels will increase energy demands in the heart in order to maintain pumping effectiveness. Whatever the primary cause of the energy imbalance, chronic decreased ATP levels will impede  $\text{Ca}^{2+}$  re-uptake from the cytoplasm into the sarcoplasmic reticulum by the  $\text{Ca}^{2+}$  ATPase, SERCA2a, and so lead to  $\text{Ca}^{2+}$ -related activation of hypertrophic and arrhythmic pathways.<sup>60,61</sup>

### DCM – a 'cytoskeletopathy'...

Elucidation of the molecular underpinnings of DCM (Table II) has been more intractable than for HCM, perhaps reflecting the greater complexity in terms of familial and environmental causes of the former disorder. Unlike HCM, which is a genetic disorder in the majority of cases, only a minority (about 30%) of patients with DCM have evidence of familial clustering. Even less commonly, DCM is a feature of syndromic disorders, often with accompanying skeletal and limb-girdle myopathies. Interestingly, it was this co-existence of DCM and skeletal myopathy in Duchenne's and Becker's muscular dystrophies that led to the discovery of dystrophin (Fig. 1) defects as a cause of pure X-linked DCM, without overt skeletal involvement,<sup>62,63</sup> and to the consequential speculation that, much as HCM is a 'sarcomeropathy', familial DCM is a 'cytoskeletopathy'.<sup>64</sup> Additional studies in other skeletal myopathy phenocopies of DCM, which implicated more proteins that make up the internal structure of the cell, the cytoskeleton (Fig. 1), strengthened this proposal. Furthermore, it was not only the internal cytoskeleton that was responsible, because proteins that form part of the extracellular matrix function in cell:cell contact at myocyte junctions ( $\beta$ - and  $\delta$ -sarcoglycan, desmo-

plakin; Fig. 1),<sup>65</sup> proteins that stabilise the membrane around the cellular nucleus (lamin A/C, emerin; Fig. 1)<sup>66,67</sup> and proteins that connect these elements (desmin; Fig. 1)<sup>68</sup> were also found to be defective in patients with dilated hearts (Table II). Moreover, the discovery that a number of cytoskeletal proteins form substrates for proteases expressed by viruses known to cause cardiac dilation<sup>69,70</sup> may imply that the aetiological principle involved in DCM may be instability at any structural point throughout the integrated substructure of the cardiac syncytium.

Very recently, several of the sarcomeric protein-encoding genes originally implicated in HCM (Table II, Fig. 1), have also been found to be defective in some DCM cases, blurring the lines of aetiological distinction between these two disorders.<sup>71,72</sup> It may be that these particular mutations cause DCM rather than HCM because they involve different functional domains of these proteins, or simply because the sarcomere itself, although primarily a functional unit in the myocyte, inherently also forms part of the integrated internal structure of these cells.

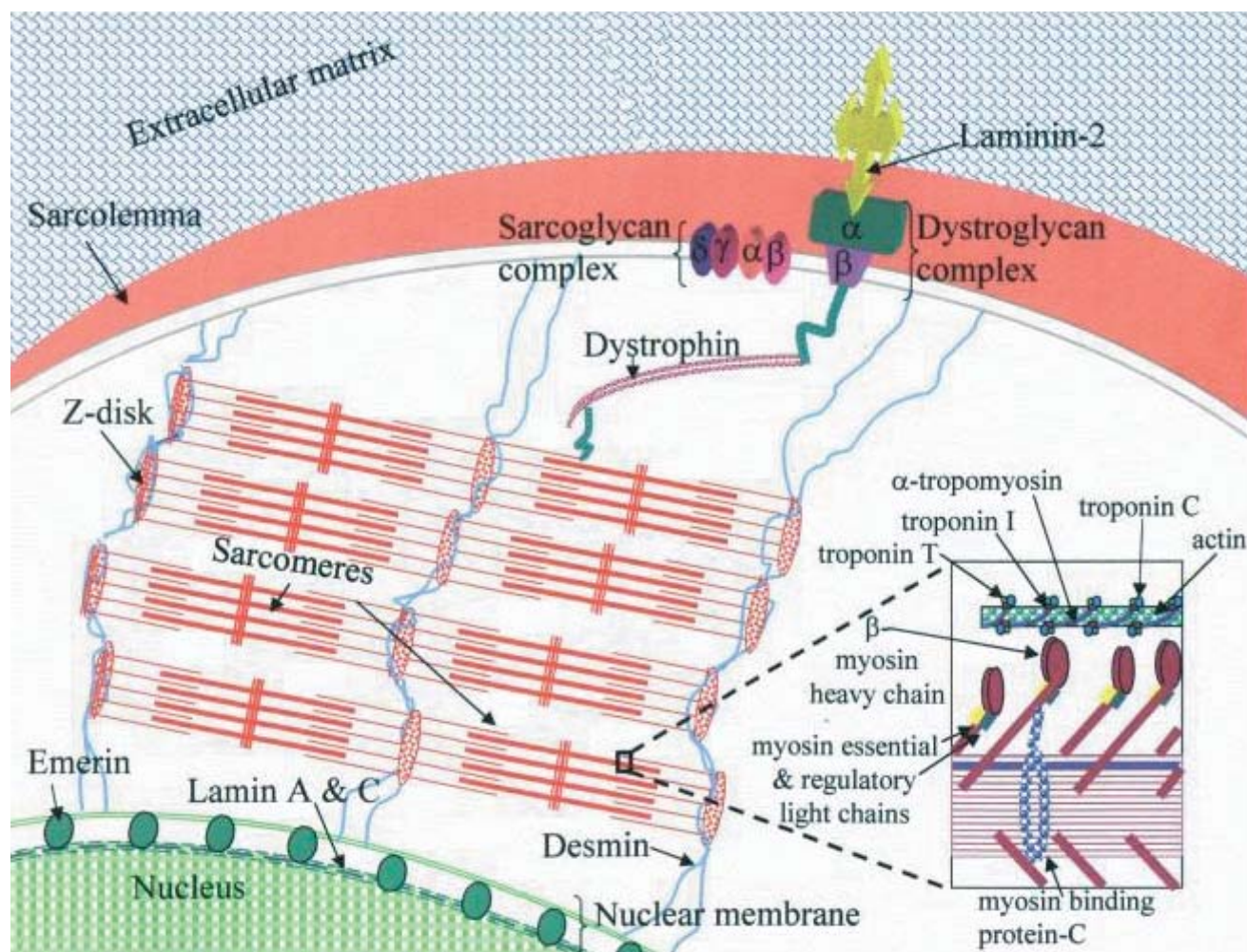
### ...with energy metabolism involvement?

However, to add to the complexity, it seems that DCM is also not purely a disease of cell architecture, but that energy metabolism could play a major role here as well, as is now postulated for HCM. It has long been known that mitochondrial DNA defects have been associated with DCM,<sup>73–76</sup> however, it has been difficult to prove whether these mitochondrial defects are the cause or consequence of the cardiac phenotype. Recently, though, Barth syndrome, a DCM phenocopy disorder, was found to be caused by defects in the gene encoding the enzyme tafazzin, which results in a failure to produce a specific glycerophospholipid.<sup>77,78</sup> As this lipid forms part of the inner mitochondrial membrane, mitochondrial dysfunction and therefore reduced energy supply is implicated as the cause of DCM. This finding is interesting in the light of data from previous morphometric studies of mitochondria in biopsies from DCM and HCM hearts, which suggested that the mitochondria of DCM hearts showed decreased activity, while those from HCM hearts showed increased activity.<sup>79</sup>

Many familial DCM-causing genes are currently only localised to particular chromosomal regions and not yet identified. However, the discovery of the responsible genes may be facilitated by combining our new understanding of the structural/architectural and energetic aetiological principles underlying DCM with the deluge of genetic data emanating from the human genome project. Consequently, it can be anticipated that pinpointing DCM-causing genes may well enter the fast track, in parallel with developments in HCM gene identification through the last decade.

### ARVC – myocyte death?

Although ARVC was particularly slow to reveal its aetiological secrets, with originally some speculation only but no concrete proof concerning the involvement of viruses, its



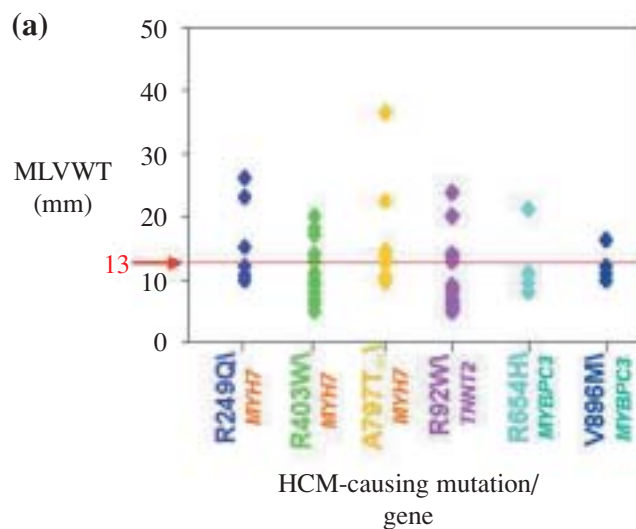
**Fig. 1. Cellular localisation and interactions of proteins involved in HCM and DCM. Schematic representation of a section through part of a cardiac myocyte, illustrating the position and interactions of many of the various proteins that have been implicated in HCM and/or DCM.**

familial nature became clear in time and lead to some elucidation of its aetiology (Table II). Defects in the ryanodine receptor were implicated as the cause of pure ARVC,<sup>80-82</sup> while studies of the molecular cause of Naxos disease, a complex phenocopy of ARVC, pointed to structural proteins whose role is to maintain stability of the cardiac desmosomes.<sup>83</sup> Initially the involvement of the former protein may seem to imply a different molecular mechanism than that proposed for Naxos disease. However, the ryanodine receptor regulates the release of  $\text{Ca}^{2+}$  from the sarcoplasmic reticulum, and failure to do so may lead to cell death by  $\text{Ca}^{2+}$  overload. Similarly, destabilisation of the desmosomes may also cause cell death, unifying these apparently divergent aetiological principles for ARVC, and perhaps providing the explanation for the extensive myocyte loss seen in ARVC. To date, there is no clue as to why these dead cells are replaced by fibro-fatty tissue, or why the right ventricle is most severely affected. From molecular genetic studies in ARVC families, it is clear that the genes implicated so far are not the only ones responsible for this cardiac phenotype, and it is possible that with identification of more ARVC-causing genes, these two features of the disease may become more readily understood.

## Modifier effects in cardiomyopathies

As with all inherited diseases, the inherited cardiomyopathies also feature extensive variability in phenotypic expression, even between related carriers of the same disease-causing mutations. Moreover, in HCM, the same disease-causing mutations have also been associated with diverse clinical outcomes in families from different ethnic ancestry.<sup>84</sup> This indicates that additional factors, genetic or environmental, which are neither necessary nor sufficient to cause clinical disease, modulate the expression of the primary 'disease-trigger'. The identity of these modifiers remains largely unknown, although a number of factors, such as components of the renin-angiotensin system,<sup>85-88</sup> mitochondrial variations,<sup>89,90</sup> peptide hormones<sup>87</sup> and trophic factors<sup>91</sup> have been suggested. Furthermore, identification of these modifying factors is complicated by the genetic and allelic heterogeneity of the cardiomyopathies. Large-scale systematic studies, either in transgenic animals or in patients sharing the same mutation and genetic background (founder cohorts) are likely to provide the most insight into the identities of these modulators.

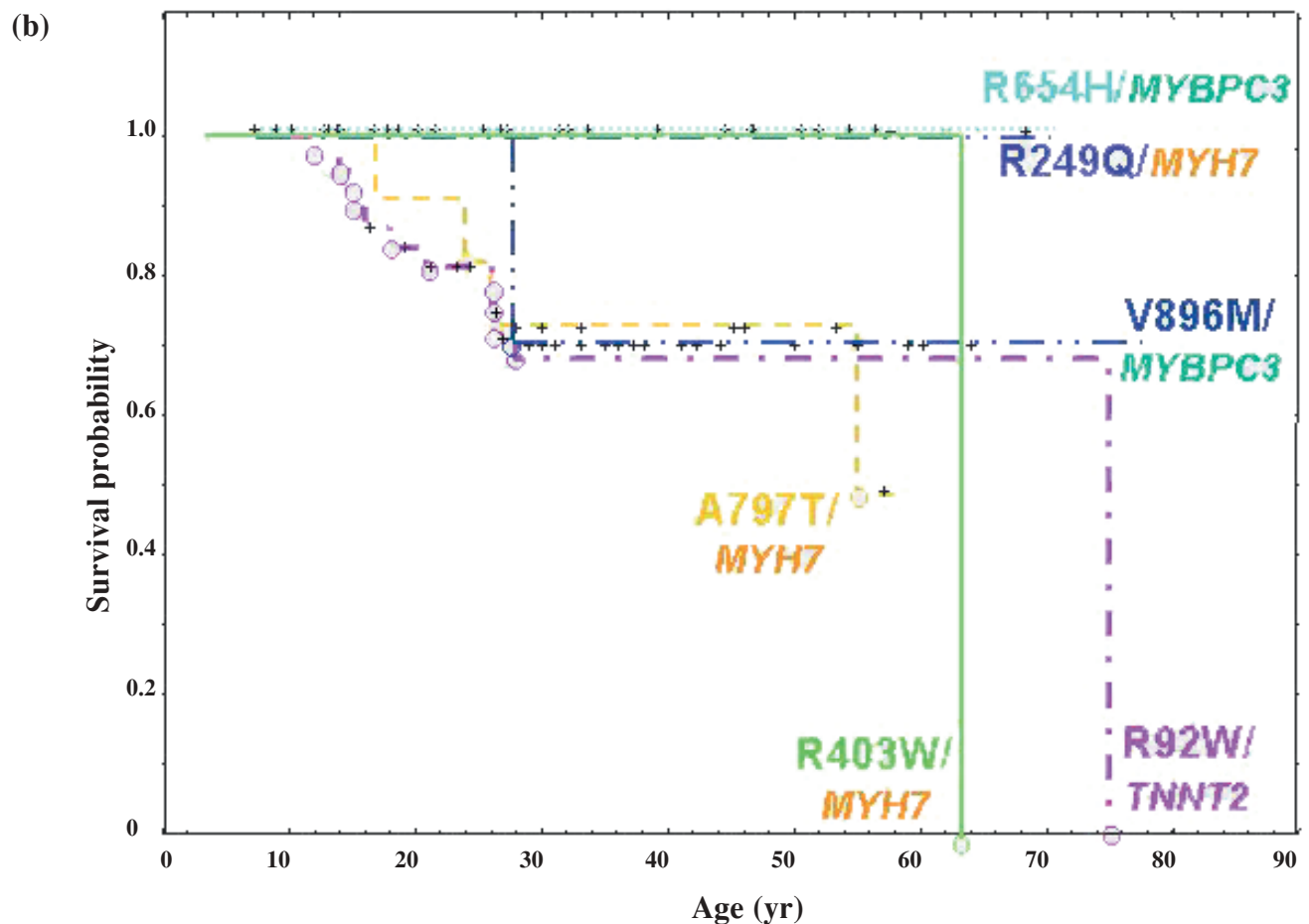




**Fig. 2. Hypertrophy and risk of sudden cardiac death in individuals carrying HCM-causing mutations.**

(a) Comparison of the extent of hypertrophy in family members with single distinct HCM-causing mutations in different sarcomeric protein encoding genes.

(b) Kaplan-Meier product limit curves for survival amongst the mutation carriers in these families. For some mutations (R403W, R92W), the majority of individuals do not meet the clinical diagnostic criterion of a maximum left ventricular wall thickness (MLVWT)  $\geq 13$  mm [indicated by arrow in (a)], yet they can still be at risk of early sudden cardiac death (R92W). Troponin T – R92W; cardiac  $\beta$ -myosin heavy chain – R249Q, R403W, A797T; cardiac myosin binding protein-C – R654H, V896M.



### Impact of genetic knowledge on patient management and treatment

Even though our knowledge on the genetic aetiology of the inherited cardiomyopathies is still incomplete, it is clinically relevant.<sup>15,92</sup> Although not all cases of cardiomyopathy will be due to inherited genetic defects, the frequency of familial forms has highlighted the need for a complete and detailed family history, as well as for clinical follow-up and counselling of all first-degree relatives of newly diagnosed cardiomyopathy patients. It has become clear that a much lower diagnostic threshold is appropriate when interpreting diag-

nostic tests in first-degree relatives of affected patients, particularly in family screening for HCM.<sup>15</sup> Where DNA-based genetic diagnosis is practicable, it allows early detection of individuals at risk; this may be particularly relevant in cases where a mutation is associated with a subtle clinical phenotype but a significant increase in risk of SUD. However, it should be emphasised that, because of the aetiological heterogeneity underlying the cardiomyopathies, genetic diagnosis is currently most feasible for the inherited cardiomyopathies and in a family setting. Diagnosis is still mostly performed at a research level by research institution laboratories acting as referral centres.\*



## Conclusion

Our understanding of the pathogenesis of cardiomyopathies has increased dramatically over the last few decades, due to the identification of some molecular causes of the inherited form of these diseases. Elucidation of the full spectrum of genetic contribution to the development and progression of the cardiomyopathies represents a new challenge in the study of these diseases and will undoubtedly lead to new therapeutic approaches in the not-too-distant future.

\*Information about relevant laboratories in South Africa is available from the corresponding author, Johanna Moolman-Smook.

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